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A Primer on Ranaviruses . . . . . David S. Lee 141

What You Missed at the October Meeting . . . . . John Archer 149

Herpetology 2013 152

Unofficial Minutes of the CHS Board Meeting, October 18, 2013 155

News and Announcements 155

Advertisements 156

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A Primer on Ranaviruses

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If you are a turtle, a frog or even a tadpole you need to read this—it's really scary!

Forget vampires and zombies. There is a real cold-blooded killer out there, a pathogen named *Ranavirus*. As the label implies, it is not exactly frog friendly, but it also causes illness and death in salamanders, reptiles and fish. This virus is now found worldwide. Transmission is rapid and can result from either direct or indirect contact with infected animals. The virus enters cells of the host and takes over the cell processes for its own replication. Ranaviruses can infect multiple cell types and cell death can occur in as little as nine hours, quickly leading to loss of organ function. Susceptibility varies with species. In some frogs, for example, mortality can result in just three days. Experiments done on infected and uninfected salamanders showed the virus could be transmitted when the salamanders were in contact for as little as one second (Brunner et al., 2007). Exposure to water or soil contaminated with *Ranavirus* can also result in disease.

History and background

Ranaviruses are believed to have evolved in fish and only later began to infect amphibians and reptiles (Jancovich et al., 2010). This genus of viruses was first reported from amphibians in the 1960s in a population of northern leopard frogs, *Lithobates pipiens* (Granoff et al., 1965), yet the impact of widespread virus-related die-offs was not recognized until the 1990s. Research experiments demonstrate that the virus can be transmitted within and between three classes of vertebrates.

Since the mid-1990s *Ranavirus* has been taking a devastating toll on native populations of reptiles and amphibians across the U.S. Especially hard hit are frogs, toads, salamanders and their larvae, as well as turtles. Hundreds of thousands of these animals have died from the lethal virus and the disease continues to spread. The cause of the sudden appearance of a global plague from this pathogen is uncertain, but possibilities include trade in food and ornamental fish, reptiles, amphibians, and/or its emergence from unknown reservoir hosts resulting from changes in the environment.

Ranavirus has been documented as being responsible for amphibian die-offs, some of them massive events, in over 20 states. To date over 85 species of turtles and amphibians have been involved with the die-offs, where mortality can range from a few individuals to thousands. In some cases where amphibian breeding sites support a number of frogs and/or salamanders the die-offs involve multiple species. Ninety-four percent of the known cases of *Ranavirus* have been reported since 1998. While to some degree this represents a growing awareness of the problem, this figure strongly suggests that the virus is becoming more common and widespread.

Die-offs of amphibians have been reported on private, state and federal lands, including several national parks and wildlife refuges. To date, most of the species involved are relatively common, but the virus has also caused problems for populations of threatened and endangered species. The reasons for the emergence of *Ranavirus* in wild populations vary from site to site. Often there are stress-related elements, man-made or natural, associated with outbreaks. Disturbance of the site and/or pollution are important but not necessarily required factors. The virus exists in aquatic habitats: ponds, lakes, permanent wetlands and vernal amphibian breeding sites. Turtles and breeding adult amphibians moving into recently flooded wetlands are likely carriers of the disease. A common factor in *Ranavirus* outbreaks is the rapid drying of wetlands. This apparently is because the drying concentrates turtles and amphibians and accelerates metamorphosis. The immune system of amphibians is suppressed during transformation to the adult stage, increasing the chance of pathogen infections, and disease.

Ranaviruses are members of the Iridoviridae, a group of double-stranded DNA viruses. There are six recognized species and numerous strains, however in North America viruses related to the *Ambystoma tigrinum* virus (ATV) and Frog virus 3 (FV3) appear to be the most important to reptiles and amphibians. The Bohle iridovirus (BIV) from Australia is also of concern. Some ranaviruses may be able to infect more than one class of vertebrates (e.g., amphibians, reptiles and fish). The incubation period is variable—five days to several weeks. The virus was identified in skin, intestines and kidneys of African clawed frogs, *Xenopus laevis*, within three hours of introducing them to infected water (Robert et al., 2011). Ambient temperatures, dose of virus exposure, immunosuppression, the host's developmental stage, and species differences in susceptibility to various *Ranavirus* strains probably affect infection timing. Ranaviruses replicate only at temperatures between 12 and 32°C; because of this, birds and mammals are not suitable hosts (Chinchar, 2002). The virus can remain viable in frozen fish for over two years (Langdon, 1989).

Three genera of viruses of the family Iridoviridae affect fish. Ranaviruses and megalocytiviruses are pathogens that have recently appeared. Both types cause severe disease outbreaks, occur globally, and affect a broad spectrum of hosts. The hematopoietic necrosis virus from Australia was the first *Ranavirus* found to cause epizootic mortality in fish. Like other ranaviruses it lacks host specificity. A distinct but closely related virus, European catfish virus, occurs in fish in Europe, while very similar ranaviruses appear in fish and amphibians in Europe, Asia, Australia, North America and South America. These viruses can be distinguished from one another and this could

allow policies of the World Organisation for Animal Health (OIE) to minimize their spread. However, at this time limited information and variations in disease expression create difficulties in sampling strategies, and there remains uncertainty surrounding the taxonomy of some ranaviruses (Whittington et al., 2010).

All types of amphibians including salamanders, newts, frogs and toads are susceptible. Larvae and metamorphic stages are most often associated with massive mortality events. Adult amphibian morbidity and mortality are reported less often, but have been observed in the wild, as well as in captivity. Some species may have covert infections and be able to shed and transmit virus to other susceptible animals without ever exhibiting clinical signs. Likewise, nonlethal infections have been documented and it is likely that these latent infections explain the persistence and emergence of the disease in both wild and captive populations. Ranaviruses found in fish, amphibians, and types of other reptiles may serve as reservoirs for susceptible chelonians.

Ranavirus is but one of a number of viral pathogens that have been reported in turtles. The two important viral diseases of freshwater and terrestrial chelonians are herpesvirus disease in tortoises (multiple clinical signs and high mortality may occur) and iridoviral (*Ranavirus*) disease. See Origgi (2006) for a review of herpesvirus disease of tortoises and Jacobson (2007) for a general and comprehensive review of chelonian viral diseases. Recently, occurrence of *Ranavirus* in lizards has also been documented (Stöhr et al., 2013).

Johnson et al. (2008) report affected species that included captive Burmese star tortoises, *Geochelone platynota*, a free-ranging gopher tortoise, *Gopherus polyphemus*, free-ranging eastern box turtles, *Terrapene carolina carolina*, and a Florida box turtle *Terrapene carolina bauri*. They also found evidence for *Ranavirus* infection in archived material from previously unexplained mass mortality events of eastern box turtles from Georgia in 1991 and Texas in 1998. *Ranavirus* infections were also found in sympatric species of amphibians at two locations with infected chelonians. The profiles of *Ranavirus* isolated from a dead Burmese star tortoise and a southern leopard frog, *Rana utricularia* (= *Lithobates sphenocephalus*), found nearby, were similar. These findings support the ideas that certain amphibians and chelonians are infected with a similar virus and that different viruses exist among different chelonians. Amphibians may serve as the major reservoir host for susceptible chelonians. This study also demonstrated that significant *Ranavirus* infections are likely more widespread in chelonians than previously suspected.

Eastern populations of tiger salamanders (*Ambystoma tigrinum tigrinum*) are listed at some level of conservation concern in almost every state in which they occur; in most they are considered endangered. Most populations are isolated, disjunct, and both their overall numbers and range are declining. Titus and Green (2013) reported *Ranavirus* in populations of tiger salamanders on Long Island. Thus, the threat of this virus to populations of endangered species is no longer to be considered as just a potential one, it's real.

Death from the virus is not pretty. In amphibians the disease is likened to Ebola or epizootic hemorrhagic disease due to body swelling and hemorrhaging. Hemorrhagic lesions are characteristic of fish with *Ranavirus* infections and often in reptiles as well. Tissue necrosis is extensive because the virus commandeers multiple cell types.

Recognition

Infection does not always cause disease. Long-term non-clinical carriers have been identified. Clinical signs vary depending on the host and a number of other factors.

In infected **fish** the hematopoietic tissue is usually severely affected. General pathological signs include pale gills and liver, friable kidneys and livers, and ecchymosis and petechiation on ventral body surfaces. Fish with *Ranavirus* often exhibit no external symptoms. Both fresh and saltwater species are affected, and the virus can be spread to animals eating live, dead, or previously frozen infected fish.

In **amphibians** ranaviral outbreaks can result in sudden onset of illness; in a wetland often hundreds or thousands of sick individuals are seen over a one- to five-day period. Overall mortality rates in larvae and juveniles will exceed 90%. A good indication of disease is lethargic animals swimming erratically and weakly, or on their sides. Infected frogs and salamanders typically have subtle to severe hemorrhages on the ventral surface, particularly at the base of the hind limbs, and around the vent. In some cases hemorrhages are present from the chin to the tip of the tail; at other times they may appear in specific sites or as irregular patches. The abdomen may also become enlarged and reddened (redleg-like symptoms) and amphibians may have skin ulceration and/or epithelial proliferation. Mild to severe fluid accumulations can appear under the skin of the abdomen and hind legs. Hemorrhaging also occurs in multiple tissues, especially the liver, kidney, heart tissue, and digestive tract. Red-tinged or clear fluid accumulations may appear in the body cavity.

Turtles infected with this virus show overall weakness, swollen eyelids, exhibit discharge from the mouth and nose, and the tongue and palate often show dull white or thick yellow plaques. In some cases turtles may have ulcers on the bottoms of their feet. Conjunctivitis and subcutaneous edema of the eyelids and neck have also been noted. Mortality is high and other clinical signs can include pharyngeal ulcers, skin sloughing, and marked lethargy (Duncan, 2011). White dissection plaques can also be found in the pharynx and esophagus. Infections spread throughout the body affecting many organs, including blood vessels. Other studies show consistent lesions in affected turtles included necrotizing stomatitis and/or esophagitis, fibrinous and necrotizing splenitis, and multicentric fibrinoid vasculitis. Intracytoplasmic inclusion bodies were rarely observed in affected tissues (Johnson et al., 2008). In terrestrial turtles lesions are perhaps more difficult to detect as they are primarily in the oral cavity and associate with internal organs (typically respiratory and gastrointestinal), but can also include eye and nasal discharges. Aquatic turtles exhibit hemorrhages and ulcers, with the ulcerations occurring along respiratory and digestive tracts. Death results from organ dysfunction and secondary infection by other pathogens.

Potential Impacts

There is no question that *Ranavirus* outbreaks are now common and the virus is widespread. Allender et al. (2013) examined 606 eastern box turtles from across the southeastern United States and found a 1.3% prevalence of *Ranavirus*. There was a higher infection rate in juveniles than adults, but the difference was not significant. This seemingly low percentage of infected box turtles is misleading, and the authors of the study suggest that the low detection prevalence is a result of the quick time from exposure to development of the disease and death of the turtles. This would mirror the findings for amphibian *Ranavirus* mortality with the difference being the sick and dead amphibians are more easily identified due to their seasonal concentrations at breeding sites.

In that a number of our endangered and threatened species have restricted distributions and survive in relatively small populations, they are potential targets for extinctions resulting from ranaviruses. So too are numerous peripheral populations of reptiles and amphibians, many of which are state-listed as species of conservation concern. Species endemic to specific springs and spring runs, those confined to narrow elevation zones on isolated mountains, and fish and turtles whose distributions are limited to single drainage systems would seem very vulnerable. In the latter case infected bait-fish released by fisherman could contaminate independent drainage systems with novel ranaviruses. Subterranean species of blind cave-dwelling fishes and salamanders are at risk. Aquatic cave-dwelling animals typically live at very low population levels and entire underground aquatic systems could quickly succumb to the virus. D. S. Lee (1969) reported on the occurrence of bullfrogs in pools deep within cave systems, and other types of amphibians commonly inhabit the twilight zones of caves. Both represent potential avenues of *Ranavirus* transport into subterranean systems where the cool ambient temperatures of cave systems would prove favorable to the virus.

The spread of *Ranavirus* to sites harboring isolated amphibians could result in loss of subpopulations. Narrow-range endemics, as well as relict, disjunct and peripheral populations are vulnerable, and could be quickly extirpated. Distant transport of the pathogen by contaminated boots, field equipment, or release of infected animals to biologically significant remote sites is a real concern. This is exacerbated by the different strains of *Ranavirus*, and their ability to infect a wide spectrum of hosts. Over time the emergence of novel viruses could occur across a broad landscape. Furthermore, roads, pipelines, and development have fragmented landscapes to the point that if isolated populations of even common and widespread species are extirpated, natural recolonization is unlikely to occur. Nonetheless, development induced isolation does not fully protect sites from exposure to *Ranavirus* as there are a number of anthropomorphic dispersal mechanisms for the virus.

People maintaining captive collections of turtles and tortoises outdoors run the risk of locally occurring amphibians infecting them. Native frogs frequently take up residence in outdoor pools set up for aquatic turtles, and even a single infected frog could easily contaminate an entire collection. Additionally, *Ranavirus* can survive in fresh and frozen fish. This is another means for the disease to infect facilities maintaining captive turtles.

How we are likely aiding and abetting

This virus can remain viable outside a host for 30 days or more (Nazir et al., 2012). Boots and field equipment that come in contact with water and sediments contaminated with *Ranavirus* can later spread the pathogen to other areas. This is also likely to occur with the chytrid fungus (*Batrachochtrium dendrobatidis*) that affects amphibians. The spread of these diseases is also the result of visitation to wetlands for recreational activities. Studies conducted in the Great Smoky Mountains National Park found a higher *Ranavirus* prevalence in salamanders at sites with high public access. Additionally, livestock and agricultural pesticides in wetland areas stress hosts increasing the likelihood of *Ranavirus* outbreaks (Gray et al., 2007).

The release of individual captive animals is an ongoing problem. Virus-infected pets, both commercially purchased and wild-caught captives, can harbor ranaviruses asymptomatically, and the serendipitous broadcasting of the disease to native species is a major concern. While some states have regulations forbidding the release of captive reptiles and amphibians into the wild, they are almost impossible to enforce. Additionally many well-intended people and organizations translocate animals to new localities as natural habitats are lost to development. This is yet another avenue for the unintended dispersal of *Ranavirus*. Attention needs to be focused on wildlife rehabilitation centers. Often their goal is to help sick animals resolve their health issues so they can eventually be released. Fortunately the staffs of the centers can be trained to identify clinical signs and reptiles and amphibians can be tested for the virus prior to release.

Establishment of exotic species may also add to the problem. *Ranavirus* has recently been documented in *Anolis* lizards in Florida (Stöhr et al., 2013). South Florida is probably the exotic *Anolis* capital of the world. Any exotic species comes with a potential for introducing novel pathogens.

Fish hatcheries and other commercial aquaculture practices can provide an environment for rapid adaptation of *Ranavirus* strains. Studies have shown that ranaviruses collected from hosts raised and maintained in captive facilities, such as bullfrog farms and bait stores selling minnows, were more virulent than those found in wild populations (Storfer et al., 2007; Hoverman et al., 2011). The appearance of this virus in Japan is suspected to have originated from captive-raised frogs being released into the wild (Une et al., 2009).

The number of fish hatcheries, fish farms, and commercial facilities where people pay to catch farm-raised fish in the United States is phenomenal. In North Carolina alone sales of farm-raised freshwater fish exceeds $16.5 million. On a worldwide basis 47% of the food fish consumed are farm raised. Hatcheries run both by federal and state agencies, as well as those managed by the private sector, present some major issues. The hatcheries overseen by wildlife agencies maintain their facilities for stocking streams and lakes for fishermen, while private hatcheries sell their fish to individuals wishing to stock

private farm ponds. In both cases the fish are dispersed widely, providing the potential for rapid wholesale spread of *Ranavirus*. Nelson (2010) reported *Ranavirus* from two ponds at Harrison Lake National Fish Hatchery in Charles City County, Virginia. Based on this the author then examined tadpoles from four warm-water fish hatcheries in Virginia to determine if they were infected with *Ranavirus*. The virus was detected in tadpoles in three of the four warm-water Virginia hatcheries. Temperature and the length of time a pond is filled with water were significant predictors of the proportion of tadpoles that tested positive for *Ranavirus*. Similar results were found by Nelson over multiple years. Obviously precautions should be taken to ensure that ranaviruses are not spread when fish are transferred from one hatchery to another, or to the wild, but also of concern is the likelihood of the spread of the virus via the native amphibians that use hatcheries and fish farms as breeding sites.

Some garden centers that supply plants and other items for backyard outdoor pools also sell tadpoles for stocking garden ponds. While the wholesale suppliers of these tadpoles vary from store to store, clearly the stock does not necessarily come from local sources. No matter the origin of these tadpoles, as well as the fish and aquatic plants offered for sale from the same display containers, they are potential dispersal agents for *Ranavirus*. In addition biological supply companies supply tadpoles for classroom use so that students can witness metamorphosis, often the young frogs are released after they transform. Maryland has posted a warning about this practice as it relates to the spread of *Ranavirus* and other diseases on their Natural Resources web site (http://www.dnr.state.md.us/wildlife/Plants_Wildlife/herps/catalogue_frogs.asp).

The release of unwanted "minnows" and salamanders used as fishing bait is yet another avenue for spreading the virus. Lee and Knight (1968) described the commercial sale of native salamanders for fishing bait in the eastern United States in the 1960s. While for the most part the commercial aspects of this are no longer in effect due to current wildlife regulations, the noncommercial practice continues. The appearance of *Ranavirus* outbreaks in the central United States was attributed to the sale and use of infected tiger salamander larvae (*Ambystoma tigrinum*) for fishing bait (Ridenhour and Storfer, 2008). This salamander is also widely used as bait in the southwestern states.

This, of course, leads to the question as to the extent of infections being spread from large lots of turtles distributed to domestic and foreign retail stores from our southeastern turtle farms. They annually market over 200,000 hatchling turtles, mostly red-eared sliders, within the United States, and sell 10 million overseas. In addition there are turtle farms where turtles are raised for meat, and others specializing in exotic and other high-end species for sale to the hobbyist. Due to the nature of turtle farming, where large numbers of adult turtles are maintained in overcrowded conditions where breeding stocks constantly are being supplemented with additional wild-caught turtles, outbreaks of ranaviruses are likely. The fact that many turtle farmers use scraps from commercial catfish farms and similar aquaculture sources increases the opportunity for contamination from other facilities. It has been demonstrated that novel strains have developed in various aquaculture facilities (e.g., bullfrogs, Hoverman et al., 2011; bait store fish, Gray and Miller, 2013) but to date commercial turtle farms have not been tested. Furthermore simply moving infected individuals long distances and releasing them can result in the emergence of novel ranaviruses (Ridenhour and Storfer, 2008).

Robert et al. (2007) identified African clawed frogs as a possible vector for *Ranavirus*. They found that adult frogs typically clear FV3 infections within a few weeks, but viral DNA was still present in their kidneys several months after they were experimentally infected. The virus was also detected in seemingly healthy frogs that were not deliberately infected. In this study the authors hypothesized that "covert FV3 infection" may occur in *Xenopus*. This finding and other aspects of their study suggest that FV3 can become dormant in resistant species making some species viral reservoirs. The use of African clawed frogs for this research is interesting in that during the 1950s and '60s this species was widely used for pregnancy testing. The species was imported in large numbers and shipped to clinics and hospitals throughout the country. In following decades dwarf clawed frogs, *Hymenochirus* spp., were imported and commonly sold along with aquarium fish in pet stores. The commercial global distribution of African clawed frogs is reportedly responsible for spreading chytrid fungus and accounts for the extinctions of various native frog faunas --i.e., 30 species wiped out in a Panama forest (J. J. Lee, 2013). Today there are a number of businesses, like *Xenopus* Express, that supply clawed frogs for medical use, research centers, and the pet trade throughout the country and as well as in international sales.

As pointed out earlier (D. S. Lee, 2012) *Ranavirus* could become particularly troublesome as a result of turtle races where wild caught, non-native captives, and pet store purchased turtles and tortoises, are all mixed together at the events. Some of these events are even held back to back with frog-jumping contest. While this virus is a serious concern, the potential impact on native turtle populations is but one of a number of important reasons that these turtle derbies should be restructured, if not eliminated altogether. A committee of people working in meetings for months would be hard pressed to come up with a more cost efficient and effective means than turtle derbies to spread a deadly pathogen into our native populations of reptiles and amphibians.

Add to this mix the various wholesale farm-bred fish, frogs and turtles imported from Asia and sold live as food items in Asian markets across the United States. These would prove likely vectors for ranaviruses, and possibly a source for establishing new strains of the disease in this country. A decade or so back I purchased several adult frogs from an Asian market for testing and they all were positive for chytrid fungus. This was brought to the attention of our state wildlife agency, but they were unwilling to enforce their injurious wildlife regulations due to possible ethnic backlash.

Another issue is the release of fish, turtles and frogs by Buddhists—a practice resulting from a cultural/religious history going back at least 2,000 years. Because of this, certain Buddhist sects release store purchased birds, fish, turtles and other creatures. The belief is that freeing animals back into the wild is a means of achieving blessing, and turtles and tortoises are

considered as the most karmaically valuable animals to release. The people are not particularly concerned with the survival of the animal; to receive blessings they simply buy and release them. This same practice occurs not just in Asia, but also in the U.S. and Canada with goldfish and hatchling sliders being the most common subjects for release (Maclachlan, 2011; Liao and Lee, 2012).

At other times Buddhists will purchase and release creatures when family members are sick, believing it helps with the healing process. Releases may occur daily until the person is fully recovered. This practice is feasible because of the low cost of a number of commercially available species. Twenty young sliders, for example, can be purchased in China for the equivalent of US$15 (CNY 100). The releases are not limited to hatchlings; adult and subadult sliders are often released en masse.

And let's not forget all the captive animals held in classrooms, typically individual locally caught creatures, brought in by students and held on display for the remainder of the school year. Usually they are assigned to some student to release prior to the summer recess.

Diagnosis, Testing and Treatment for *Ranavirus*

Polymerase chain reaction (PCR) is the most useful test and is becoming more widely available. Real-time PCR techniques allow detection of smaller amounts of virus, but to identify the group type (ATV or FV3 virus-like) of *Ranavirus* present, conventional PCR with DNA sequencing is required. Determining the specific species of *Ranavirus* usually requires cell culture, virus isolation, and molecular characterization. These techniques are not widely available outside of research laboratories. Conventional PCR can provide false-positive results if confirmatory DNA sequencing or Southern blot analysis is not performed. Histopathology is helpful to screen for lesions in sick animals, but lesions tend to be nonspecific unless intracytoplasmic inclusion bodies are seen. Virus isolation, immunohistochemistry, transmission electron microscopy, cell culture, and serology (not widely available or validated for most species) have also been used to identify infected animals (Duncan, 2011).

For laboratory analysis the best choice for tissue samples are ones collected at necropsy, especially liver, kidney and skin (if lesions are present). Frozen tissues are required for virus isolation and are generally best for molecular analysis as well, however, freezing does not work for histology. For histology, tissues should be submitted fresh or fixed in 70% ethanol or 10% neutral buffered formalin. Ethanol-preserved tissues may be used for some molecular testing. Formalin-fixed tissues may also be used for some molecular testing if the length of time in formalin is minimal (days to weeks). It is possible to perform PCR on paraffin-embedded tissues. Samples can also be collected from clinically ill animals via cloacal or pharyngeal swabs, tissue biopsy (tail clips), or blood samples. Plastic handled, rayon-tipped swabs are preferable for collection of PCR samples. If living animals are tested, results should be interpreted with caution, recognizing test limitations—a positive test result is more reliable than a negative result. Test sensitivity for antemortem PCR increases with time post-exposure and development of clinical signs of illness (Duncan, 2011). Individual laboratories can provide more information regarding screening.

While *Ranavirus* outbreaks are typically fatal, Wack et al. (2013) developed protocols to treat diseased turtles and prevent the virus from spilling over to other captive animals at the Maryland Zoo. Their work resulted in the survival of 14 of 27 captive eastern box turtles after an outbreak of *Ranavirus* in the summer of 2011. Their methods included strict quarantine guidelines, modified environments, intensive care—including nutritional support, and extensive multimodal medical treatment by the zoo's veterinary staff. The surviving turtles all successfully overwintered, far exceeding previous survival rates for box turtles with this virus. Hausmann et al. (2013) used 11 of these turtles to determine if they had developed an immunity to the virus. Seven turtles were inoculated with a dose of the same strain of the virus and four controls were injected with an equal volume of saline. The turtles were monitored for 9 weeks. Only one of the re-infected and none of the controls died. Except for the turtle that died, the inoculated turtles showed only minor signs of the virus, suggesting that the turtles acquired some level of immunity from their earlier exposure. The single box turtle that died exhibited intracytoplasmic inclusion bodies in the kidney, lungs, pancreas, liver, and vas deferens; vasculitis in the spleen, pancreas, lungs and liver; nephritis; pneumonia; esophagitis; hepatitis; and enteritis. (It appears that the virus gained access to the zoo's outdoor box turtle exhibit by a visitor adding an additional turtle to the group. When the exposed turtles were brought in for treatment, one additional, previously unmarked, individual was discovered in the group.)

Quantitative tests have been developed that are 100% effective in detecting frog virus (FV3) in turtles. FV3 DNA can be identified in whole blood samples, oral swabs and cloacal swabs. Clinical indications of viral infections seen in experimentally infected red-eared sliders include lethargy, conjunctivitis, oral plaques and ulcers, while those in box turtles were fractures and diarrhea. Treatment with anti-viral therapy is reported to have poor success. Red-eared sliders, *Trachemys scripta scripta*, that were experimentally exposed to the FV3 virus had higher mortality rates when maintained at 22°C than at 28°C, suggesting that ranaviruses are less successful at higher temperatures. Analysis of infected box turtles showed a single oral dose of valcyclovir to have a positive effect, and that it may prove to be useful against the virus (Allender et al., 2013).

Precautions and actually doing something about this

Education regarding handling, maintaining, breeding, transporting, and selling farm-raised fish, bait fish, ornamental fish for outdoor ponds, and wild and captive bred pet trade reptiles and amphibians will become increasingly important. Restoration projects and stream and lake stocking that involve release of fish, reptiles and amphibians will need to verify that the released animals are free of the virus. In addition, field biologists, and the general public alike will need to be aware of the issues caused by ranaviruses. It is important that wildlife biologists working for government agencies, zoological facilities and wildlife rehabilitation centers understand the threat posed by ranaviruses and take proactive roles in preventing further spread.

Monitoring subsets of wild populations and captive collec-

tions of turtles and amphibians would be beneficial to track and control the spread and extent of this virus. Populations of rare and endangered species deserve special attention and commercial imports and shipments of fish, reptiles and amphibians, particularly ones reared en masse on farms and shipped in wholesale quantities for retail sales to the public, need to be regularly checked for the disease.

Partly as a result of concerns about *Ranavirus* outbreaks a number of state agencies have started taking a close look at turtle races. Maryland's DNR has made it known that turtles and frogs entered in race events may not be released back into the wild once the races are over. Both Pennsylvania and Maryland have started enforcing the illegal entry of state protected species (in Pennsylvania this includes box turtles) in turtle races. In part, based on *Herp Digest*'s Internet circulation of concerns about these races (D. S. Lee, 2012), several race sponsors canceled races altogether (e.g., see Moss, 2013b). At a number of events turtle advocate organizations are screening turtles to help insure that visibly sick and diseased turtles are not entered in the events, or allowed to have contact with other turtles. In the summer of 2013 a number of race sponsors across the country were approached by conservation organizations and asked to alter the way the races are currently conducted or to plan alternate events (e.g., Moss, 2013a). The Tortoise Reserve has information prepared by the veterinary community on the various reptile diseases that could be spread by turtle race activities. This is available to individuals or organizations interested in educating the various race sponsors via the Tortoise Reserve. Subsequently the Center for Biological Diversity started contacting sponsors of turtle races explaining their unintended consequences and suggesting that they modify the way races are conducted. Prior to the 4th of July turtle race in BelAir, Maryland, the Susquehannock Wildlife Society posted an online commentary about the problems the annual race was causing native wildlife asking the race sponsors to suspend the event in future years (http://www.daggerpress.com/2013/06/30/susquehannock-wildlife-society-calls-on-public-to-leave-wildlife-in-the-wild-this-fourth-of-july/). The majority of the comments posted were quite supportive of the Society's position, but it is interesting to read the mind-sets of some of the people commenting on this post who strongly believe that such traditional events should not be altered.

Preventing the spread of this virus will be taxing for those of us working daily with captive reptiles and amphibians. Disinfection of supplies, equipment, water dishes and caging that come in contact with the animals, or their water, is important. One-minute contact with solutions of 3 percent bleach, 0.75 percent Nolvasan® (chlorhexidine diacetate), or 1 percent Virkon® S (potassium peroxymonosulfate) are effective in killing the virus. Nolvasan is less toxic to amphibians. Disposable vinyl gloves should be rinsed, disinfected, or changed when handling different animals. While doing this is often not practical under field conditions, or when dealing with captive collections, minimally it should become standard protocol when changing field sites, or when exchanging specimens and housing between live collections. Particular care needs to be taken in and around habitats such as isolated wetlands that harbor peripheral populations, endemic species, species of state concern, and threatened and endangered species. Protocols need to be developed for people requesting access to these sites, and access will probably best be limited to those with permits and training. The release of captive amphibians and turtles will need to be limited to animals that have been tested for the virus. Stocking streams and lakes from fish hatcheries may no longer be a viable option. Additionally the sales of live fish, salamanders, frogs and turtles commercially raised on farms for bait, stocking, food and pets will need to be addressed, as possible release of these creatures into the wild by well-intended people will be difficult if not impossible to enforce. People overseeing zoos and private collections of amphibians and turtles need to be aware of the problem, particularly when acquiring new stock.

Natural resource agencies should consider conducting surveillance studies to identify infection hotspots, where ranavirus prevalence exceeds 40 percent (Hoverman et al., 2012; Gray and Miller, 2013). Once hotspots are located agencies can identify the mechanisms driving them, determine effects on populations, and come up with intervention strategies. Green et al. (2009) provide recommendations regarding sample size to detect the presence of *Ranavirus* as it relates to approximate host population size, and a 95% confidence level for detection. Large numbers of individuals from any given population will need to be tested to attain meaningful confidence levels.

It is interesting to note that the U.S. Department of Agriculture is poised to act quickly to oversee and regulate interstate movement of domestic animals and products that might possibly be infected with diseases where outbreaks can affect livestock, or spread Mediterranean fruit flies. Yet, diseases that seriously impact noncommercial native wildlife, and can be easily spread by our activities, continue to remain unchecked.

People interested in, and working with, reptiles and amphibians approach them from different perspectives. Academic researchers investigating wild populations deal with these animals quite differently than those working with them in labs. Amateur herpetologists who enjoy finding reptiles and amphibians in the field often share little in common with those who maintain captive collections of various color morphs and non-native species. Zoos, museums and nature centers are interested in educational displays, while veterinarians and wildlife rehabilitation groups focus on the health of individual animals. In addition, there are commercial collectors, exporters, importers, wholesale distributors, reptile show sponsors and pet shops. Boy Scouts working on merit badges, people rescuing turtles from roads, and retail purchasers are yet other user groups. They, along with the wildlife agencies overseeing the welfare of these animals, all need to be educated as to the plastic nature of ranaviruses if we are to have any hope of keeping this disease from becoming an outright worldwide plague.

Due to the broad, and growing, range of host species this pathogen is becoming a major threat to a significant portion of the earth's vertebrate fauna. With one in three species of amphibians and over 40% of the world's turtles already at risk of extinction the virus poses a serious additional threat to global biodiversity. Its impact likewise represents a significant problem for aquatic community composition and to the overall functioning of wetland and terrestrial ecosystems. The serious conse-

quences of this virus going, excuse the pun, viral cannot be overstated. We are witnessing a disease that can covertly breach the protective boundaries of state and national parks, wildlife refuges, and any number of private wildlife sanctuaries and similar lands that have been set aside to permanently preserve natural systems. With the potential consequences of expanding *Ranavirus* outbreaks on our native frogs and toads perhaps a follow-up book to Rachel Carlson's 1962 classic *Silent Spring* entitled *Silent Night* is now in order. A more fitting title might be *Night of the Living Dead*.

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For additional information see Manual Diagnostic Test for Aquatic Animals 2012. Infections with ranavirus. Chapter 2.1.2: 71-91, and the literature cited within [http://wahis2-devt.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.01.02_ranavirus.pdf].

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What You Missed at the October Meeting

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As I quickly glanced at the title of October's presentation I was a bit disappointed. I didn't know Jen Stabile, but I assumed she would deliver a fine talk; it was just that the title as it appeared on our website read simply "Amphibian Conservation." I know there are new members joining us frequently, but I've been a CHS member for a while and my interest in conservation had preceded my membership, so I was not looking forward to another talk rehashing facts that I already knew. If you've been even tangentially involved with amphibians and conservation you know something about the mysteries surrounding *Batrachochytrium dendrobatidis* (Bd) and the devastating effects it's having on the world's amphibians and you know that one of the biggest threats to virtually all the natural world is habitat destruction. We've had lots of speakers cover those topics in many and varied ways, and while always interesting, I just didn't feel the need for more info on the subject of amphibian declines unless we were to learn about a positive new approach to combat the declines.

Jen Stabile. Photograph by Dick Buchholz.

But if I had read the whole description of the talk I would have anticipated the meeting more eagerly. I met Jen Stabile and found her to be engaging and enthusiastic. When her opening slide appeared with the title "Biology and Conservation of the Mona Island Coqui" I started to become more interested. This wouldn't be a talk of all threats to all amphibians but rather a specific one, the Mona Island coqui (*Eleutherodactylus monensis*). And it would cover the frog's biology. Any animal's natural history is rarely boring for me. But I was still wrong in my judgment of Jen's presentation, because her talk went way beyond even her title to include natural history, a bit of travelogue, some taxonomy, a little geography, current husbandry, future directions, and a lot of humor.

Jen Stabile is the Amphibian Conservation Coordinator at the Albuquerque BioPark Zoo. In 2004 she began her professional career as a docent and intern at the Central Florida Zoo and Botanical Gardens. She interned at the Med Toxin and Venom Laboratory where she received her venomous training and in 2005 the Central Florida Zoo hired her as Reptile and Amphibian Keeper, later promoting her to Amphibian Conservation Coordinator. In 2011 she went to work in that capacity and as Senior Keeper of Herpetology for the Albuquerque BioPark Zoo. She has worked with Ray Ashton, the Florida Fish and Wildlife Research Institute, and Dr. Rafael Joglar of the University of Puerto Rico and Proyecto Coqui in Puerto Rico. She has over eight years of experience both in-situ and ex-situ working with the coqui frogs of Puerto Rico. I think her Facebook photo shows you her personality. She looks totally confident in a leather cowboy hat, plaid shirt and snake stick slung over her shoulder. Her fascination with coquis is evidenced by a tattoo on her wrist. It displays a petroglyph found in Puerto Rico representing the coqui.

She began by briefly mentioning the global amphibian crisis, highlighting the fact that the largest numbers of threatened species occur in Latin America, particularly the Caribbean. Of the 18 endemic amphibian species in Puerto Rico, three are thought to be extinct, 11 are of conservation concern and three are common. All 17 coqui species belong to the genus *Eleuther-*

One of the reasons Jen Stabile likes working with coquis (*Eleutherodactylus coqui*). They stay in one place longer than many frogs. Photograph by Gil Hidalgo.

The star of Jen's presentation, the Mona Island coqui (*Eleutherodactylus monensis*). Photograph by Jen Stabile.

A welcome sight after five hours in a small boat on rough seas. Mona Island has no airport and no permanent inhabitants. Photograph by Jen Stabile.

Mona Island has a diversity of habitats, with lush tropical forests near the base of the cliffs, very dry conditions on top of the plateau, and many caves. Photograph by Jen Stabile.

The reigning champions of Mona are the endemic rock iguanas, *Cyclura cornuta stejnegeri*. Photograph by Jen Stabile.

The lab at Albuquerque BioPark Zoo where Jen Stabile raises her coquis. The public has an excellent view. Photograph by Jen Stabile.

A hatchling Mona coqui. Jen's tattoo of the coqui petroglyph for size. Photograph by Jen Stabile.

A hatchling Mona coqui. Photograph by Jen Stabile.

odactylus. We saw pictures of the three extinct species and Jen explained that *Eleutherodactylus* translates as "free-toed"; the frogs have no webbing between their toes. Like many of that genus, coquis are all direct developing. Tiny, fully formed frogs hatch out of the eggs, which are often guarded and defended by the males. She had nice pictures of males guarding eggs and hatchlings. A new coqui species, *Eleutherodactylus juanriveroi*, was named as late as 2007. The common coqui, *E. coqui*, can be found throughout the island of Puerto Rico, even in the dry cactus-strewn habitats that Jen showed in photos.

And they've been introduced in Hawaii, where they are disparaged as not only an invasive species but also one with a loud and annoying call. Jen finds the call pleasant, as probably do most Puerto Ricans, who have lived with the frogs and their calls from birth. Puerto Ricans adore the little frogs, as evidenced by the ubiquity of references to the coqui, from coqui candy (" . . . made probably not *from* coqui frogs, but named *after* coqui frogs") to coqui earrings to coqui key chains. Jen said that the coqui is a national symbol of Puerto Rico that insinuates itself into the culture to a greater extent than the bald eagle does ours. Myths abound about the frog. One has a prince turned into a coqui to sing for his people and another that coquis cannot be removed from the island or they will stop singing or even die. The ancient people of Puerto Rico, the Taíno, left many petroglyphs of the frogs.

Jen then moved to her journey to the island of Mona. Maps placed the four- by seven-mile island about halfway between Puerto Rico and the island of Hispaniola in the infamous, at least to seafarers, Mona Passage. The island is a small national reserve with no permanent inhabitants surrounded by frequently rough seas and deep cold water. Five hours of waves crashing over the bow of the small boat that was her transportation made Jen really appreciate stepping onto the small beach area around the dock on Mona. A limestone island mostly surrounded by cliffs, the lower beach areas looked lushly tropical in her pictures, but the plateau on top of the cliffs was covered with sparse xeric plants and what looked like rocky soil. The contrast between the two habitats was amply illustrated by her pictures. While mentioning that pirates had apparently used the island, Jen flashed a picture of Johnny Depp as Captain Jack Sparrow on the screen and expressed her disappointment in not finding him or the supposed treasures buried on the island. She did find other interesting stuff, as illustrated by her pictures of the endemic anole and gecko, interspersed with photos of caves, spiders, bugs, and beautiful beaches.

Even though the the systematics and evolution of the genus *Eleutherodactylus* have been extensively studied, little is known about their natural history and biology, and of all the Puerto Rican *Eleutherodactylus*, *E. monensis* is probably the least known. The endemic frog is the only amphibian on the island. It is terrestial and is listed as vulnerable. Working with Dr. Joglar, Jen was on Mona to try and add to that limited knowledge. They worked in three sites covering a cave, a bromeliad quadrant on top of the plateau, and a well. Observations were focused on abundance, size, and reproduction of the frogs. Visual assessments were conducted day and night. Since this trip was in the dry season, most frogs were found in the caves and around the well, and virtually all frogs, since they are nocturnal, were found at night. We saw pictures of the sites, the frogs, and petroglyphs. A series of stills showed a coqui attacking and briefly seizing a gecko. The frogs are known to be fiercely territorial and Jen was enraptured by this demonstration of their boldness.

Because of her success in breeding other coquis, Jen had a permit to capture five males and five females to attempt captive breeding. She set up the animals in pairs with rain systems in a laboratory at the Albuquerque zoo. After losing all hatchlings from four clutches, she had the Albuquerque water tested. Even though she was filtering all water, a trace amount of naturally occurring fluoride was found. Once she switched to calcium enriched R.O. water, she's had total success and is now the proud provider for 58 tiny frogs. Beautiful Mona coqui hatchlings filled the screen and her affection for her charges was evident in Jen's voice as she described their color variability, huge appetite, and seemingly robust health. She commented on the Mona coquis' nest building as unique among the coquis that she has studied and had a series of slides from a night vision camera to illustrate that interesting behavior.

Jen had slides of the Albuquerque viewable laboratory, happy zoo visitors, cheerful steel drummers, and smiling restaurant owners to emphasis the importance of educating people about these animals. One of her successful innovations was a Puerto Rican night at a local Albuquerque restaurant. The restaurant was Mexican, but the owners were Puerto Ricans. Once a month they had dinner with bands and the frogs and souvenirs. It was a great success, heightening awareness and raising money to supplement her grants. One of the last slides showed a Mona Island iguana (*Cyclura cornuta stejnegeri*) standing on a beach, head held high. Jen described the lift she got every morning upon finding these majestic animals lining up on the beach to catch the sun's rays. She reveled in the absolute wildness encapsulated in the lizards' bold ownership of the island.

Jen Stabile is doing impressive conservation work. She delivered a presentation full of interest, humor, and enthusiasm and containing much more than I can write here, including news about Bd. She ended with pictures of her Mona Island team from Puerto Rico and encouraged us to view the website of Proyecto Coqui, the folks who have been doing coqui research for a long time. The site is in Spanish, but will soon be translated. In the meantime, Google translator does a fine job. She had pictures of her coworkers at the Albuquerque BioPark Zoo's Department of Herpetology. She encouraged us to attend the first meeting of Caribbean PARC in May. She invited us to visit in Albuquerque. She went to dinner with us after the meeting, bringing along a guest. She was just as interesting and fun at dinner as she was at the meeting. Her guest was interesting and fun. What a privilege to interact with someone of her talents. I feel sorry if you missed it.

And I started out unenthused.

Herpetology 2013

In this column the editorial staff presents short abstracts of herpetological articles we have found of interest. This is not an attempt to summarize all of the research papers being published; it is an attempt to increase the reader's awareness of what herpetologists have been doing and publishing. The editor assumes full responsibility for any errors or misleading statements.

SEED DISPERSAL BY CROCODILIANS

S. G. Platt et al. [2013, Journal of Zoology 291:87-89] note that saurochory (seed dispersal by reptiles) among crocodilians has largely been ignored, probably because these reptiles are generally assumed to be obligate carnivores incapable of digesting vegetable proteins and polysaccharides. They review the literature on crocodilian diet, foraging ecology, digestive physiology and movement patterns, and provide additional empirical data from recent dietary studies of *Alligator mississippiensis*. Evidence of frugivory was found in 13 of 18 (72.2%) species for which dietary information was available, indicating this behavior is widespread among the Crocodylia. Thirty-four families and 46 genera of plants were consumed by crocodilians. Fruit types consumed by crocodilians varied widely; over half (52.1%) were fleshy fruits. Some fruits are consumed as gastroliths or ingested incidental to prey capture; however, there is little doubt that on occasion, fruit is deliberately consumed, often in large quantities. Sensory cues involved in crocodilian frugivory are poorly understood, although airborne and waterborne cues as well as surface disturbances seem important. Crocodilians likely accrue nutritional benefits from frugivory and there are no a priori reasons to assume otherwise. Ingested seeds are regurgitated, retained in the stomach for indefinite and often lengthy periods, or passed through the digestive tract and excreted in feces. Chemical and mechanical scarification of seeds probably occurs in the stomach, but what effects these processes have on seed viability remain unknown. Because crocodilians have large territories and undertake lengthy movements, seeds are likely transported well beyond the parent plant before being voided. Little is known about the ultimate fate of seeds ingested by crocodilians; however, deposition sites could prove suitable for seed germination. Although there is no evidence for a crocodilian-specific dispersal syndrome similar to that described for other reptiles, this review strongly suggests that crocodilians function as effective agents of seed dispersal. Crocodilian saurochory offers a fertile ground for future research.

STATUS OF SEVERAL ENDEMIC MUD TURTLES

J. Reyes-Velasco et al. [2013, Chelonian Conservation and Biology 12(1):203-208] carried out surveys in northern and central Mexico to locate extant populations of *Kinosternon hirtipes chapalaense*, *K. h. magdalense*, and *K. h. megacephalum*, evaluate their conservation status, help establish captive breeding colonies of these taxa in the near future, and collect tissues for phylogenetic studies. During 2010 and 2011, the authors were able to locate extant populations of 2 of the subspecies (*K. h. chapalaense* and *K. h. magdalense*), but *K. h. megacephalum* could not be found. They also failed to locate natural springs in a radius of approximately 60 km from the type locality (and only known locality) of *K. h. megacephalum* and believe that this turtle is extinct.

NO CHYTRID IN PRESERVED GOLDEN TOADS

K. L. Richards-Hrdlicka [2013, J. Herpetology 57(3):456-458] notes that the golden toad (*Cranopsis periglenes*) of Monteverde, Costa Rica, is arguably the "poster child" for the global amphibian decline crisis. Of the known drivers of amphibian declines, it has been hypothesized that the emerging infectious disease, chytridiomycosis, caused by the fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), led to the toad's extinction. The aim of this study was to test whether the last collected and curated *C. periglenes* specimens were infected with Bd. Fifteen preserved *C. periglenes*, three of which were the last ever collected (April 1982), were swabbed for the presence of Bd. All skin swabs were tested for Bd with a specific qPCR assay and found negative for Bd. Either the zoospore loads from the specimens fell below detection limits, or the tested specimens were not exposed to Bd at the time of collection. This study highlights the importance of collecting noninvasive, field swabs from living amphibians even those facing decline.

SALINITY TOLERANCE AMONG TADPOLES

M. E. Brown and S. C. Walls [2013, Copeia 2013(3):543-551] note that amphibians in freshwater coastal wetlands periodically experience acute exposure to salinity from hurricane-related overwash events, as well as chronic exposure associated with rising sea levels. In a comparative experimental approach, the authors examined whether seven species of anuran amphibians vary in their tolerance to changes in salinity. In a laboratory study, they exposed larval *Hyla cinerea* (green treefrogs), *H. squirella* (squirrel treefrogs), *Lithobates catesbeianus* (American bullfrogs), *L. sphenocephalus* (southern leopard frogs), *Anaxyrus terrestris* (southern toads), and *Gastrophryne carolinensis* (eastern narrow-mouthed toads) from an inland population in north central Florida, and *Osteopilus septentrionalis* (Cuban treefrog) from an inland population in southwest Florida, to acute salinity for 72 h. For each species, trials were replicated in which tadpoles were exposed to salinities of 0.2 (control), 5, 10, 12, 14, and 16 ppt. For all species, tadpoles reared in the control and 5 ppt treatments had 96.7–100% survival. No *G. carolinensis* survived at salinities exceeding 5 ppt and no individuals of any species survived in the 14 or 16 ppt treatments. For all other native species, survival at 10 ppt ranged from 46.7 to 80%, but declined to 0% at 12 ppt (except for *H. cinerea*, of which only 3.3% survived at 12 ppt). In contrast, all individuals of the invasive, non-native *O. septentrionalis* survived exposure to a salinity of 10 ppt, and survival in this species remained relatively high at 12 ppt. These results illustrate that the non-native *O. septentrionalis* has higher salinity tolerance than the native species tested, which may contribute to its invasion potential. Moreover, species commonly associated with coastal freshwater wetlands differ in salinity tolerances, suggesting that salt water intrusion due to storm surges and sea level rise may affect the species composition of these ecosystems.

DO COTTONMOUTHS BASK IN TREES?

S. P. Graham [2013, J. Herpetology 57(3):428-431] notes that a common belief in the southeastern United States is that cottonmouths (*Agkistrodon piscivorus*) bask frequently in tree branches over the water's edge and are therefore of considerable hazard for anglers and boaters. Although this notion is almost certainly based upon observations of nonvenomous watersnakes (*Nerodia* spp.), there are no quantitative data that specifically address this belief. The author analyzed a 7-yr dataset on cottonmouths from two populations (Georgia and Alabama) to determine the frequency of this behavior by cottonmouths. Behavioral and substrate information were recorded for 804 separate observations on cottonmouths. Only two of the cottonmouths were exhibiting arboreal behavior (0.25% of observations)—snakes observed over the ground or water surface on branches narrower than their own bodies—confirming that this is indeed a very rare tendency in this species and not likely to be observed by the public. Instead, cottonmouths were frequently encountered on the ground along the water's edge in their characteristic ambush posture, uncoiled in aquatic situations, or coiled on elevated platforms (e.g., hummocks, piles of driftwood, logs, beaver lodges). This study demonstrates that cottonmouths have the capacity to climb but are not likely to be observed in arboreal situations.

SURVEYING CRAWFISH FROGS

P. J. Williams et al. [2013, Copeia 2013(3):552-561] note that crawfish frogs (*Lithobates areolatus*) are a relatively widespread but under studied North American species suspected to be in steep decline. Discussions to petition this species for federal listing have begun and therefore effective techniques to survey and monitor populations must be developed. Crawfish frogs produce unusually loud breeding calls, making call surveys the most efficient way to assess populations; however, their peak breeding period lasts for only a few nights, sometimes for only one night. Automated calling survey techniques were used at two wetlands where the numbers of crawfish frog males present were known (±1%) for the entire length of the breeding season to examine detection probabilities in relation to season, time of day, weather variables, survey duration, and the numbers of males present. These data were then used to answer three simple but important questions: 1) When should researchers listen—that is, what times and under what environmental conditions should surveys for crawfish frogs take place? 2) How long should surveys last? and 3) What can call surveys tell us about the size of a population? The most supported model for detection included the quadratic relationship of time and date, a positive linear relationship with temperature, and a negative linear relationship with recent rain, while the most supported model for estimating abundance included the quadratic relationship of time and date, and call rate. Five-minute surveys should suffice during peak breeding for known large populations; 15-minute surveys with repeat visits should be used for small populations or when sampling new areas. These findings should improve manually collected (auditory) call survey efficiencies for crawfish frogs, surveys that are being organized to provide the first objective data on the status of this species across its range.

HYBRIDIZATION BETWEEN GARTERSNAKE SPECIES

J. M. Kapfer et al. [2013, J. Herpetology 57(3):400-405] note that snakes within the genus *Thamnophis* (gartersnakes and ribbonsnakes) are often found in sympatry throughout their geographic distributions. Past work has indicated that some sympatric species within this genus may hybridize, but research of this nature is limited. The authors attempted to determine whether hybridization occurs between two *Thamnophis* species native to the upper midwestern United States: common gartersnakes (*Thamnophis sirtalis*) and the Butler's gartersnakes (*Thamnophis butleri*). They sampled snakes ($n = 411$) across 26 locations in Wisconsin, including sites where both species coexist and sites where only common gartersnakes are found. They conducted genetic analyses on tissue collected from individuals field-identified as common gartersnakes or Butler's gartersnakes. To verify the results of the field-collected data, tissues were analyzed from juvenile snakes ($n = 4$) suspected to be the offspring of a common gartersnake and a Butler's gartersnake that were housed together in a captive situation. Of the field-collected snakes analyzed, eight snakes were consistent with expected common × Butler's gartersnake hybrids. All four of the captive offspring analyzed resolved as putative hybrids, corresponding with the field-collected samples. Butler's gartersnake is a globally rare species, endemic only to the upper midwestern United States. Studies involving the potential for hybridization between common and uncommon species are useful from a conservation perspective. The low incidence of hybridization observed would indicate that hybridization between these species is uncommon. Further research investigating rates of hybridization would help assess any potential threat posed by outbreeding between common and rare gartersnakes in this region of the United States.

HOG-NOSED SNAKES AVOID PAVED ROADS

L. E. Robson and G. Blouin-Demers [2013, Copeia 2013(3): 507-511] note that roads can directly impact animal populations by increasing the risk of mortality; however, a more subtle ecological effect may lie in the way roads impede gene flow by creating barriers to animal movement. The authors investigated the effect a road network, containing both paved and unpaved surfaces, has on the movement patterns of eastern hog-nosed snakes (*Heterodon platirhinos*) in the Long Point region of Ontario, Canada by radio-tracking 17 adult snakes over two years. They used telemetry data collected in the field to infer the minimum number of road crossings made by snakes, and random walk simulations to estimate the number of road crossings snakes would have made if they moved randomly in relation to roads. Comparing the inferred and expected number of crossings allowed the authors to test the hypothesis that roads constrain movements because snakes avoid crossing them. Overall, the road network did not impede snake movements. Examined separately, however, road substrates affected movement: snakes avoided crossing paved roads while they crossed sand roads readily. Male and female snakes crossed roads at the same frequency. While the risk of road mortality is reduced by road avoidance, such avoidance of paved roads may contribute to the genetic isolation and further decline of this species-at-risk.

WATERSNAKES IN CALIFORNIA

J. P. Rose et al. [2013, J. Herpetology 57(3):421-427] report that northern watersnakes, *Nerodia sipedon*, have been introduced into California's Central Valley and pose an important new challenge for the management of biodiversity in the state's already greatly distressed freshwater ecosystems. Nonnative watersnakes will likely compete with federally threatened giant gartersnakes, *Thamnophis gigas*, and prey on native amphibians and fish, including young salmonids, many of which are imperiled. Three types of aquatic funnel traps and three different methods were used to estimate the abundance and density of *N. sipedon* in a small wetland in Roseville, California. Capture rates did not differ significantly among the three trap types but snakes captured in large box funnel traps were nearly 300 mm longer on average than those captured in minnow traps. Estimates of the abundance of *N. sipedon* in the 2-ha trapping area were similar for the mark–recapture model, Leslie depletion curve, and the actual number of snakes removed over 57 days (112.4–119 individuals; approximately 56.2 snakes/ha). Extrapolating to the entire 6.2-ha aquatic area, the population likely numbered approximately 348 individuals. Several females were gravid, demonstrating successful reproduction by this species outside its native range. Captured snakes included more small *N. sipedon* compared with studies in its native range. This may be due to a sampling bias in trapping methods but more likely reflects a population growing rapidly from a few initial founders with relatively fewer large adults. The authors recommend immediate action to prevent the spread and broader establishment of *N. sipedon* across the Central Valley of California.

MAP TURTLE DIETS

T. Richards-Dimitrie et al. [2013, Copeia 2013(3):477-484] note that alterations of flow regimen, pollution, and introductions of exotic species have significantly altered the composition of invertebrates in many river systems throughout the world. How these alterations affect the diet of higher level predators is not well understood. The authors studied the diet of northern map turtles (*Graptemys geographica*) in the dam-regulated Susquehanna River in north-central Maryland. Northern map turtles are a relatively large, top-order predator that is legally endangered in the state and is impacted by commercial collecting elsewhere. Gastropods, trichopteran larvae, and invasive clams (*Corbicula* sp.) predominated across diet samples. Marked sexual and size-related differences occurred. Adult male *G. geographica* fed primarily on a group of small gastropod species (Planorbidae, Hydrobiidae, Physidae), trichopterans, and *Corbicula*, while adult females fed primarily on pleurocerid snails. There was virtually no overlap in the diets of the two sexes of *G. geographica*. This is of special conservation concern because two different groups of prey are needed in order to support this population of northern map turtles, and many North American pleurocerid gastropod species are highly endangered and also threatened by hydroelectric activity. These results are consistent with reports of other map turtle populations before the invasion of zebra mussels (*Dreissena* sp.), which often result in a sharp change in diet. Zebra mussels have already been documented immediately upstream of our study site, so impacts from this invasive species may become apparent in the near future.

LEAPING SALAMANDERS

W. G. Ryerson [2013, Copeia 2013(3):512-516] studied jumping in the Ocoee salamander, *Desmognathus ocoee*. Individuals were persuaded to jump five times over a 5-cm gap by tapping metal forceps directly behind the individual. Unlike most terrestrial vertebrates, which use force generated from the hind limbs to jump, salamanders jump by laterally bending and then rapidly straightening the body, using momentum to carry the individual through the air. This movement is strikingly similar to both the terrestrial escape response of mudskippers and terrestrial blennies, and shares a general pattern of movement with the C-start escape response in several aquatic vertebrates. While the axial musculature appears to be responsible for this behavior, it remains to be seen what role the limbs and tail play. Across a twofold range in body sizes, few kinematic parameters were correlated with size. The lack of strong scaling relationships suggests a spring mechanism that allows performance to be maintained despite a twofold increase in size.

DICHROMATISM IN NORTHERN MAP TURTLES

G. Bulté et al. [2013, Chelonian Conservation and Biology 12(1):187-192] note that sexual dichromatism is common in many animal taxa, but little quantitative information on sexual dichromatism is available for turtles. The authors quantified sexual dichromatism in the postorbital spots of northern map turtles (*Graptemys geographica*) using reflectance spectrometry and examined the relationship between postorbital spot coloration and circulating testosterone among males. The coloration of postorbital spots was found to differ between the sexes, with adult males exhibiting brighter spots than adult females. However, adult males and juvenile females did not exibit siginficant differences in coloration, and testosterone levels did not explain the variation in postorbital spot coloration among males.

OVIPOSITION SITES OF SPOTTED SALAMANDERS

M. M. Kern et al. [2013, J. Herpetology 57(3):445-449] note that oviposition site selection is an important aspect of reproduction for species such as amphibians that breed in dynamic environments. They examined predictors of oviposition site selection of spotted salamanders (*Ambystoma maculatum*) at Cowan's Ford Wildlife Refuge in Mecklenburg County, North Carolina. They conducted egg mass surveys and checked 40 minnow traps for potential predators every 2 days along transects, sampling a subset of all representative habitat within the wetland. Random points were generated for each egg mass to compare microhabitat conditions in areas where egg masses were present versus undetected. Statistical methods were used to rank the associations between oviposition site and water depth, temperature, predator density, and vegetation. *Ambystoma maculatum* egg mass location was best predicted by deeper water and denser submergent vegetation. The results suggest that *A. maculatum* select oviposition sites actively, rather than ovipositing in all available microhabitat, implying that vegetation structure and hydrology of ephemeral wetlands are important for the successful reproduction of this species.

Unofficial Minutes of the CHS Board Meeting, October 18, 2013

The meeting was called to order at 7:45 P.M. at the Schaumburg Public Library. Board members Josh Baity and Cindy Steinle were absent.

Officers' Reports

Recording Secretary: The minutes of the September 13 board meeting were read, discussed and accepted.

Treasurer: The September treasurer's report was given, discussed, and accepted.

Membership Secretary: Numbers are holding at just under 500. The list of expiring memberships was read.

Vice-president: Cindy Steinle was absent. The holiday meeting, to be held on Thursday, December 26, was discussed. Mike Dloogatch moved to allocate $200 for food at the holiday party, Aaron LaForge seconded, the motion passed unanimously. Stephanie Cappiello will be organizing the refreshments.

Corresponding Secretary: Stephanie has been having problems with the phone. She is working on resolving the issues.

Publications Secretary: Aaron has updated the Grants page to announce the 2014 program.

Committee Reports

Shows:
- Notebaert Nature Museum, first full weekend of each month.
- 3-in-one Expo, Arlington Park Racetrack, November 9–10.
- SEWERFest, November 10.

Old Business

Junior Herpers: Cards will be printed with the dates for next year's meetings. Rich Lamzsus is working on name badges for staff and kids. Discussion about membership cards and coloring sheets led to the need for more operating cash. Jenny moved to allocate $300 for the running of Junior Herpers for the next 6 months. Jim seconded, the motion passed unanimously.

Nominating Committee: The slate is ready for the elections in November.

Web Site Update: Barbara Nieri Hood is working on the update.

Salamander Safari: Jason is looking into possible locations.

CHS 50th Anniversary: Dick Buchholz thinks he can get a donation of an articulated alligator skeleton for the Midwest Herpetological Symposium auction

New Business

Symposium Sponsorship: The Hoosier Herp Society is looking for sponsors for the MHS next year. $100 puts a logo on the symposium T-shirt. Mike Dloogatch will look into this.

NARBC: Andy will write reimbursement checks for those who worked the CHS booth at NARBC and paid the entrance fee.

December board meeting: The Malawys will again host the December board meeting. Aaron moved to allocate $200 for refreshments, Jenny seconded, and the motion passed unanimously.

Round Table

Rich Lamzsus adopted a Mexican black kingsnake, bred it and successfully hatched babies.

Mike Scott got two new snakes at NARBC.

Aaron got pink-tongued skinks at NARBC.

John Archer had patches made with the CHS logo. He can get more, or have shirts embroidered with the logo. He will offer them at the next meeting.

Dick Buchholz commended John for the patches. Dick explained how he got a new hedgehog through Bob from animal control. Happy birthday, Dick!

Barbara is really proud of the Junior Herp Society and the staff!

The meeting was adjourned at 9:22 P.M.

Respectfully submitted by recording secretary Jenny Vollman

News and Announcements

FUNDING AVAILABLE FOR PRAIRIE RESEARCH

Prairie Biotic Research (PBR) is an all-volunteer nonprofit that fosters basic biotic research in prairies and savannas. PBR funds grants up to $1000 to individuals for the study of any grassland taxon anywhere in the USA. They support both natural history and experimental science, and are especially eager to support independent researchers (those lacking institutional support), but anyone may apply. Since 2002, they've awarded 155 grants worth $148,946 to people in 32 states. Many of these grants supported graduate student research.

In 2014, at least 10 grants of up to $1000 each will be funded by PBR, including some restricted by donors to support research in IA, IL, MI, MN, ND, SD and WI. Visit prairiebioticresearch.org to learn more. Check out the history and overview files in the Small Grants section of the website to see what sorts of proposals have won funding in the past.

Those who won funding in 2013 are ineligible for this funding in 2014, but those who won funding longer ago are welcome to submit proposals to further that same work or to support a new project. You must have a U.S. Social Security number to apply and the research for which you seek support must be done in the USA. Proposals are due by December 31, 2013.

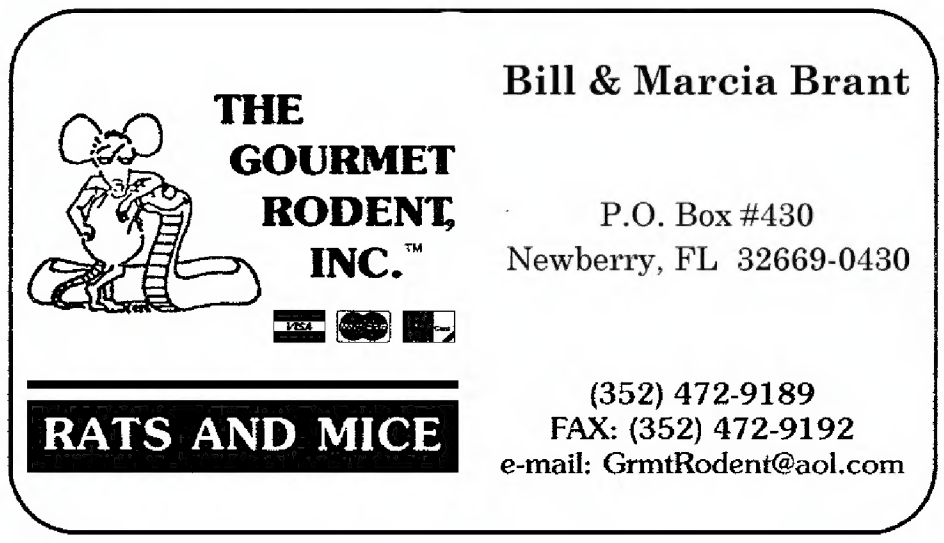
Advertisements

For sale: rats and mice—pinkies, fuzzies and adults. Quantity discounts. Please send a SASE for pricelist or call Bill Brant, *THE GOURMET RODENT*, PO Box 430, Newberry, FL 32669-0430, 352-472-9189, E-mail: GrmtRodent@aol.com.

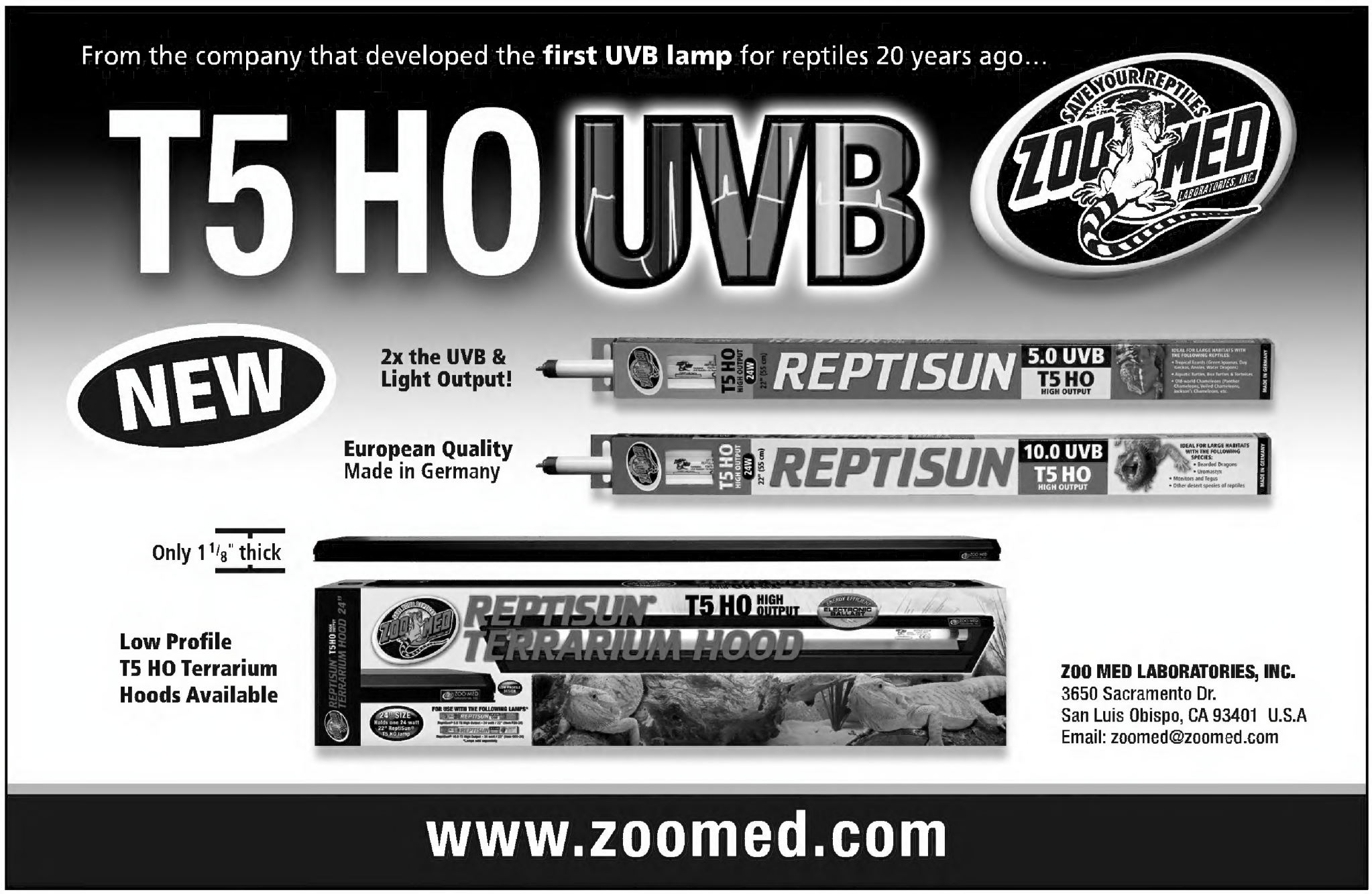
For sale: **highest quality frozen rodents**. I have been raising rodents for over 30 years and can supply you with the highest quality mice available in the U.S. These are always exceptionally clean and healthy with no urine odor or mixed in bedding. I feed these to my own reptile collection exclusively and so make sure they are the best available. All rodents are produced from my personal breeding colony and are fed exceptional high protein, low fat rodent diets; no dog food is ever used. Additionally, all mice are flash frozen and are separate in the bag, not frozen together. I also have ultra low shipping prices to most areas of the U.S. and can beat others shipping prices considerably. I specialize in the smaller mice sizes and currently have the following four sizes available: Small pink mice (1 day old—1 gm) , $25 /100; Large pink mice (4 to 5 days old—2 to 3 gm), $27.50 /100; Small fuzzy mice (7 to 8 days old—5 to 6 gm) , $30/100; Large fuzzy mice / hoppers (10 to 12 days old—8 to 10 gm), $35/100 Contact Kelly Haller at 785-234-3358 or by e-mail at kelhal56@hotmail.com

For sale: High quality, all locally captive-hatched tortoises, all bred and hatched here in the upper midwest. Baby leopards, Sri Lankan stars, and pancakes usually available, and are all well-started and feeding great! Leopards are $125 ea., Sri Lankans (2012 hatched) $475 ea. And Pancakes are $195 ea. Leopards for out of state sale/shipping require a veterinary health certificate (inquire for cost). E-mail at KKranz1@wi.rr.com or call Jim or Kirsten at 262 654 6303.

Herp tours: **Costa Rica herping adventures**. Join a small group of fellow herpers for 7 herp-filled days. We find all types of herps, mammals, birds,and insects, but our target is snakes. We average 52 per trip, and this is our 10th year doing it. If you would like to enjoy finding herps in the wild and sleep in a bed at night with air-conditioning, hot water and only unpack your suitcase once, instead of daily, then this is the place to do it. Go to our web-site http://hiss-n-things.com and read the highlights of our trips. Read the statistics of each trip and visit the link showing photos of the 40 different species we have found along the way. E-mail at jim.kavney@gmail.com or call Jim Kavney, 305-664-2881.



Line ads in this publication are run free for CHS members—$2 per line for nonmembers. Any ad may be refused at the discretion of the Editor. Submit ads to mdloogatch@chicagoherp.org.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UPCOMING MEETINGS

The next meeting of the Chicago Herpetological Society will be held at 7:30 P.M., Wednesday, November 27, at the Peggy Notebaert Nature Museum, Cannon Drive and Fullerton Parkway, in Chicago. This meeting will include the annual election of officers and members-at-large of the CHS board of directors. Also at this meeting **Stephen L. Barten, D.V.M.**, will speak about field herping in southern Illinois, Wisconsin and southwest Texas.

The December meeting will be a holiday party. Because the last Wednesday is Christmas Day this party will take place on Thursday evening, December 27. The CHS will provide soft drinks and snacks. If you would like to bring something edible to share with the group, you are invited to do so. If you would like to bring an animal to show off to the group, you are encouraged to do that as well. This will be a chance to socialize all evening and get to know your fellow members a little better.

The regular monthly meetings of the Chicago Herpetological Society take place at Chicago's newest museum—the **Peggy Notebaert Nature Museum**. This beautiful building is at Fullerton Parkway and Cannon Drive, directly across Fullerton from the Lincoln Park Zoo. Meetings are held the last Wednesday of each month, from 7:30 P.M. through 9:30 P.M. Parking is free on Cannon Drive. A plethora of CTA buses stop nearby. Free parking is available in the walled-in area to the south of the loading dock ramp.

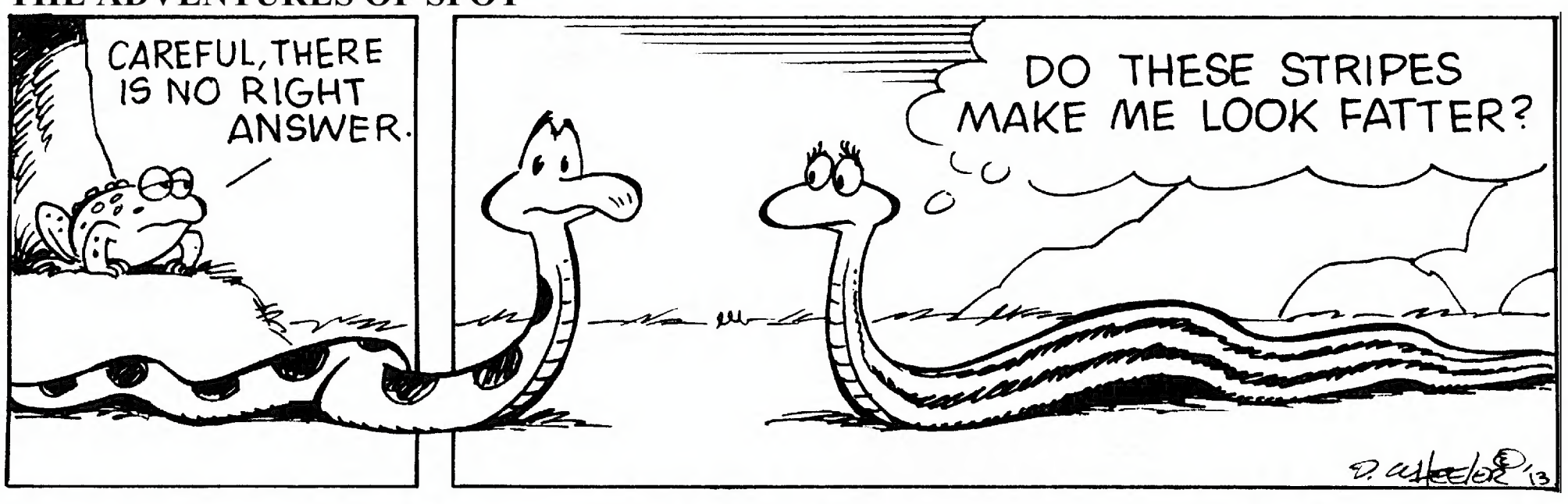
Board of Directors Meeting

Are you interested in how the decisions are made that determine how the Chicago Herpetological Society runs? And would you like to have input into those decisions? If so, mark your calendar for the next board meeting, to be held at 7:30 P.M., December 13, at the home of Linda and Andy Malawy in Naperville. If you wish to attend, please call Linda at (630) 717-9955.

The Chicago Turtle Club

The monthly meetings of the Chicago Turtle Club are informal; questions, children and animals are welcome. Meetings normally take place at the North Park Village Nature Center, 5801 N. Pulaski, in Chicago. Parking is free. For more info visit the group's Facebook page.

THE ADVENTURES OF SPOT



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